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**Patentanmeldung Nr. Patent application No. Demande de brevet n°**

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**PRIORITY DOCUMENT**

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Process for the preparation of a water continuous acidified emulsion and product obtainable by the process

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**Process for the preparation of a water continuous acidified emulsion and product obtainable by the process**

**Field of the Invention**

The invention relates to a process for preparing a water continuous spreadable acidified food product comprising fat and protein that is stable to cyclic temperature changes. The invention also relates to a food product that is a water continuous emulsion that comprises fat and protein that is stable when the product is subjected to cyclic temperatures changes between that of storage at refrigeration temperatures and exposure to ambient temperatures when being used.

**Background Art**

Water continuous emulsions as food products have been prepared starting with fat and have been used as spreads on a variety of food products like on bread and toast. The consumer prefers that the spreadable product maintains its firm shape when stored at refrigeration conditions as well as when taken out for use at ambient temperatures. The product should also have a soft consistency such that when it is applied on a soft surface like a bread, it should spread easily and not tear the bread. The source of the fat for preparing such products are of diary, vegetable, or marine origin. The protein contributes to the texture of such products and proteins have been used from dairy source like milk and from vegetable source like soy and pea. Processes to prepare such products have been described in the prior art.

WO97/08956 (Unilever, 1997) describes a vegetable and dairy fat based spread that comprises fat of which 10-55% is a non-dairy fat, up to 4.5% milk protein, gelatin or gelatin replacer and

optionally up to 1% structuring agent and having a pH in the range of 4.6 to 5.2 and a Stevens firmness value of 200-500 g at 20°C and a value of 50-250 g at 20 °C. The process for preparing this comprises the steps of pasteurising the desired mixture by heating to high temperature, cooling to a culturing temperature, adding an acidifying bacteria to acidify the mixture to the desired pH, heating the mixture to above 60 °C to inactivate the culturing organisms, followed by homogenizing the mixture at 50-600 bar, preferably at a temperature higher than 60°C followed by filling in container and cooling to desired temperatures.

WO03/043430 (Unilever, 2003) describes a food product having a dispersed oil phase and a continuous aqueous phase, the product comprising 5 to 40% fat of either dairy, vegetable or marine source, from 0.05 to 15% protein and 0.01 to 3% biopolymer said product having a pH in the range of 3.7 to 5.8. The product is prepared by a process comprising the steps of preparing the aqueous phase, mixing the aqueous phase with fat, heating to a pasteurisation temperature, homogenising the mixture at a temperature above the melting point of the fat, acidification to the desired pH and followed by one more step of homogenisation.

International Dairy Journal 12 (2002) 889-897 describes experiments where model oil-in-water emulsion were prepared. Experiments were conducted where the samples were heated before homogenisation and also where samples were heated after homogenisation. However the samples were prepared close to neutral pH (6 to 8).

The consumer often stores the product, so prepared, in the refrigerator (which could be from about 1 to 12 °C) for

ensuring extended use and takes the product out for consumption when the product warms up to ambient temperatures (of about 20 to 30°C). After some of the product is used, the container is placed back in the refrigerator. This temperature cycling could occur many times over the time the contents of the container are used up. It has been observed that the products prepared by the processes of the prior art, which contain crystalline fat, when subjected to such cyclic temperature changes show an increase in firmness which is evidenced by change in droplet size, measured as $d_{3,2}$. This has a negative impact on the spreadability of the product. Furthermore they are more susceptible to syneresis.

The present inventors have now surprisingly found that products that are prepared by a specific sequence of process steps are much more stable to the cyclic temperature changes that the product undergoes during its extended use.

It is thus an object of the present invention to provide for a process to prepare water continuous acidified emulsions that can be used as spreads and are stable to cyclic temperature changes that the product undergoes during use.

It is another object of the present invention to provide for an improved process to prepare said emulsions which in addition to having the desired property of stability under cyclic temperature conditions can be processed in the manufacturing units of the prior art with minimal modifications and therefore making the processing highly cost effective.

It is yet another object of the present invention to provide for water continuous acidified emulsions comprising fat and protein which can be used as a spread and in addition to having

the most important consumer attributes of the prior art food products are also stable to cyclic temperature changes.

**Summary of the invention**

The first aspect of the invention provides for a process for the preparation of a water continuous acidified emulsion comprising 10 to 50% partly crystalline fat and protein, said process comprising homogenizing, heating and acidifying wherein the process comprises the sequence of homogenising, at a temperature less than 60 °C, a water continuous emulsion comprising fat and protein where the percentage denatured protein is less than 20%, heating to a temperature and for sufficient time to prepare an emulsion with percentage denaturation of protein of more than 20% and acidifying the emulsion to a pH less than the pH of gelling of the protein having the highest pH of gelling in the emulsion.

Another aspect of the invention provides for a water continuous acidified emulsion prepared by the first aspect of the invention.

The invention also provides for a water continuous acidified emulsion comprising from 10 to 50 % fat, and from 0.1 to 6 %protein, having a pH in the range of 5.8 to 3.8 wherein the $d_{3,2}$ value does not change by more than 25% over three temperature cycles, each cycle consisting of four hours at elevated temperature and twenty hours at storage temperature, after an initial storage during 1 week at storage temperature. The storage temperature is in the range 1-12 °C (and includes common fridge temperatures) and the elevated temperature is in the range 20-30 °C (and includes typical ambient temperatures).

**Detailed description of the invention**

For avoidance of confusion, all parts and percentages are in percentage by weight of the composition unless specified otherwise.

The invention provides for a process for the preparation of a water continuous acidified emulsion comprising 10 to 50% fat and protein, said process comprising homogenizing, heating and acidifying characterized in that the process comprises the sequence of homogenising, at a temperature less than 60 °C, a water-continuous emulsion comprising fat and protein where the percentage denatured protein is less than 20%; heating to a temperature and for sufficient time to prepare an emulsion with percentage denaturation of protein of more than 20% and acidifying to a pH less than the pH of gelling of the protein having the highest pH of gelling in the emulsion.

An aspect of the present invention provides a process for the preparation of a water continuous acidified emulsion. The emulsion is preferably used as spreads on products like bread toast and crackers or as a semi-solid acidified cream. The emulsion essentially comprises fat and protein dispersed in an aqueous medium. The process for preparing the product comprises the steps of homogenizing, heating and acidifying. The sequence of process steps is important in achieving the desired product properties.

The water continuous emulsion of fat and protein could be dairy based or could be prepared using other non-dairy fat and protein. Dairy based emulsions include milk, milk concentrate and cream. Alternately, an emulsion could be prepared by known

methods started with butter-fat, water and protein. The fat used for this invention comprises crystalline fat which is defined hereinbelow.

It is preferred that the process of the invention comprises use of an edible non-dairy fat for example vegetable fat. The vegetable fat could be selected from any edible source but it is preferred that the fat is chosen from coconut oil, palm oil, palm kernel oil, soyabean oil, rapeseed oil, sunflower oil, safflower oil or hydrogenated products thereof.

Fat is present in the emulsion as prepared by the process of the invention from 10 to 50% more preferably from 15 to 35%.

The fat is preferably mixed with protein to prepare a water continuous emulsion of fat and protein. The protein used is any edible protein source but is preferably chosen from one or more of protein like milk protein, soy protein, or pea protein. The milk protein is highly preferred due to its taste and is preferably chosen from milk, skim milk powder, butter milk powder, butter serum powder, whey, whey powder concentrate, whey protein isolate or caseinate. The most preferred proteins are whey, whey protein isolate or whey protein concentrate. Protein is preferably present at 0.1 to 6%, more preferably from 1 to 4% in the product as prepared by the process of the invention. It is essential that the protein used in preparing the emulsion as per the invention has a percent denatured protein content less than 20%, preferably less than 15%. The method by which extent of denaturation is measured is described below.

Ferreira, Mendes and Ferreira (Analytical Sciences 17 (2001) 499-501 describe a HPLC/UV method to analyze proteins in dairy-type products, which has been adapted for the present purpose.

The chromatographical analysis was carried out using a gradient pump (Shimadzu; LC-10Ai) and degasser (Shimadzu; DGU-14A). Gradient elution was carried out with a mixture of two solvents: Eluent A: 0.04% trifluoroacetic acid in Milli-Q water; Eluent B: 0.04% trifluoroacetic in acetonitrile/Milli-Q water (95/5); Flow: 1 ml/min. The elution profile is given in the table below. Auto injector (Shimadzu; SIL-10AD) with a 5 µl injection volume. The analysis time is 32 minutes.

| [illegible] | [illegible] | [illegible] |
|---|---|---|
| 0 | 90 | 10 |
| 8 | 63.1 | 36.9 |
| 18 | 53 | 47 |
| 23 | 48 | 52 |
| 27 | 48 | 52 |
| 28 | 90 | 10 |
| 32 | 90 | 10 |

The analytical column (Hamilton) was filled with a Polymeric Reversed Phase (PRP-1) column containing a polystyrene-divinylbenzene copolymer-based packing; 150 * 4.1 mm; 100 Å. The temperature of the column was controlled using a Column Thermostat (Separations; Mistral) at 50°C. The effluent was monitored using a UV/VIS detector (Shimadzu; SPD 10A): λ1: 214 nm, λ2: 280 nm, Range: 1.0, Aux range: 2.

A preferred method to achieve the desired extent of denaturation is to ensure that the emulsion before the step of homogenisation is not heated to a temperature greater than 60 °C.

The water continuous emulsion of fat and protein, is then homogenized. It is an essential aspect of the present invention that the homogenisation is carried out at a low temperature, preferably below the temperature at which there is substantial denaturation of the protein, but above the melting temperature of the fat. A suitable temperature is 60 °C. Without wishing to be bound by theory, it is believed that the denaturation of the proteins should be effected by heating the emulsion after the homogenisation step thereby allowing the formation of a protein layer around the fat droplet before this layer is strengthened through the formation of intermolecular disulphide bonds resulting in a more stable emulsion under the cycling temperature conditions. The homogenisation step is preferably carried out in the range of 5 to 400 bar, more preferably in the range of 50 to 400, and most preferably in the range of 100 to 350 bar.
The product after homogenisation is heated to a temperature high enough and for sufficient time to ensure that at least 20% of the proteins are denatured, more preferably at least 30% and even more preferably at least 40%. The extent of denaturation of a protein is a function of the temperature it is subjected to and the time over which the protein is held at the high temperature (see e.g. Dannerberg and Kessler, Journal of Food Science 53 (1988) 258-263). The temperature/time dependency is different for each type of protein.

Hence a judicious choice of temperature and time of heating is chosen to achieve the desired degree of denaturation of the protein. Preferably the homogenized emulsion is heated to a temperature greater than 70 °C, more preferably to a temperature greater than 80 °C to achieve the desired result. The time of heating is preferably more than 2 minutes more

preferably more than 5 minutes and most preferably from 5 to 120 minutes.

The heated sample is then acidified using any known method of acidification to a pH below the pH of gelling of the protein. The method by which the pH of gelling of the protein is measured is described below.

The gelling pH can be determined by preparing a protein solution at the same concentration as the water phase of the emulsion. Other relevant parameters, such as salt concentration should be kept identical to the water phase composition of the emulsion as well. The slowly dissociating acid glucono-delta-lactone (GDL) is added in a concentration such that the change in pH is slow at the gelling pH of the protein. The exact amount depends on the protein sample, but a typical value would be 1% GDL in a 3% protein solution. Immediately after addition of the GDL, the solution is loaded in a stress-controlled rheometer (e.g. Carrimed AR1000) and the storage and loss moduli G′ and G″ are measured for a small oscillation amplitude (in the linear viscoelastic regime, strain typically $10^{-3}$). If tan delta = G′/G′ decreases to unity, the pH of the solution at this point is taken as the gelling pH of the protein solution.

When more than one protein are used in the process of the invention, the acidification is done to a pH below the pH of the protein having the highest pH of gelling.

When a protein like whey protein is used, the pH of gelling is about 5.8 and so in this case, the preferred pH over which the emulsion is acidified is about 5.8 to 3.8. The acidification could be carried out using acidifying bacteria or a chemical acidifying agent. When acidifying bacteria are used, the

emulsion is cooled to the culturing temperature. When the desired pH is achieved, the emulsion may be heated to a high temperature preferably above 60 °C to inactivate the culturing bacteria.

It is preferred that the process of the invention is carried out using a chemical acidifying agent. When a chemical acidifying agent is used, the agent could be added at any temperature and preferably the temperature to which the emulsion is heated is not changed before adding the chemical acidifying agent. The chemical acidifying agent is any food grade agent which can effect lowering of the pH to the desired range. Preferred acidifying agents are citric acid, hydrochloric acid and acetic acid.

Optionally the product may be subjected to a further heating step and/or homogenisation step after the acidification step in any order.

Preferably a preservative could be added at any stage of the process to ensure a longer shelf life of the product. Any food grade preservative could be added, the preferred ones being potassium sorbate, nisin and acetic acid.

The invention also provides for a water continuous emulsion comprising fat and protein as prepared by the process of the invention.

The invention also provides for a water continuous acidified emulsion comprising fat and protein, having a pH in the range of 5.8 to 3.8 wherein the $d_{3,2}$ value does not change by more than 25% percent over three temperature cycles, each cycle consisting of four hours at 25 °C and twenty hours of 5 °C.

The fat is present in this emulsion in the range of 10 to 50% more preferably from 15 to 35%. Protein is present from 0.1 to 6% preferably from 1 to 4%. It is particularly preferred that the protein used is whey protein. The $d_{3,2}$ value is measured as described below.

O/w emulsions were filled to a height of 15 mm in NMR tubes of 10 mm diameter, and thermally equilibrated for 30 min at 20 °C. A restricted diffusion-based droplet size was obtained by means of restricted diffusion through pfg-NMR using a Bruker Minispec MQ20. The details of the technique are discussed by Goudappel et al (Journal of Colloid and Interface Science 239, (2001) 535-542). A measurement yields values for the volume weighted geometric mean diameter $d_{3,3}$ and the width of the droplet size distribution when plotted as a function of the logarithm of the diameter $\sigma$. These parameters can be converted to the surface weighted mean diameter $d_{3,2}$ using the relation $d_{3,2}=d_{3,3}\cdot\exp(-\sigma^2/2)$. Measurements were carried out in triplicate and results are expressed in terms of average $d_{3,2}$ values. Definitions of droplet sizes are given by Alderliesten (Particle and Particle Systems Characterization 7 (1990) 233-241, and ibid 8 (1991) 237-241).

The fat should be partly crystalline, i.e. the fat is a mixture of crystalline fat and liquid oil. For the present invention, this means that the fat should have $1\%<N_t<99\%$ for at least part of the temperature range $1°C<t<30°C$. The measurement of $N_t$ is explained below. Preferably, the solid fat content of the product is at least 40%, preferably more than 50%, and most preferably more than 65% at storage temperature (preferably 5°C) and between 0.1 and 10% at elevated temperature

(preferably 25°C). The solid fat content of the product at various temperatures is measured as described below.

Method to determine solid fat content

The solid fat content (%) can be measured by a suitable analytical method such as NMR. The method used is low resolution NMR with Bruker Minispec apparatus. Reference is made to the Bruker minispec application notes 4,5 and 6.

The percentage of solid fat determined in bulk fat by the low resolution NMR technique is defined as the ratio of the response obtained from the hydrogen nuclei in the solid phase and the response arising from all the hydrogen nuclei in the sample. The product of this ratio and one hundred is termed the low resolution NMR solid fat content. No correction is made for variations in the proton density between solid and liquid phase. The NMR solid fat content for a sample measured at t °C is given the symbol $N_t$.

Suitable instruments adapted to determine the solid fat content are the Bruker Minispecs p20i, pc20, pc120, pc120s, NMS120 and MQ20.

Stabilization and tempering procedure was as follows:

melt fat at 80 °C

5 minutes at 60 °C

about 1 day at 0 °C

30-35 minutes at each chosen measuring temperature.

More preferably the firmness value of the product does not change by more than 25 percent over three temperature cycles, each cycle consisting of four hours at 25 °C and twenty hours of 5 °C. The procedure for measuring the firmness value as Stevens firmness is set out below:

Stevens firmness

The firmness of the products is determined by measuring the force required to penetrate a cylindrical probe in the product. The peak force (by custom expressed in gram, g; 1 g = 9.81 mN) is recorded, and averaged over triplicate measurements.

Sample height 5 cm; cylindrical probe of 0.5 inch thickness; compression rate 2 mm/s; penetration depth 20 mm. A suitable machine would be a Stable Micro Systems TA-XT2 Texture Analyzer.

The invention will now be demonstrated with respect to the following non-limiting examples.

**Examples**

Comparative Example -A:

A premix oil-in-water emulsion was prepared by mixing the following: vegetable fat ($N_5$=73%, $N_{20}$=14%, $N_{25}$=2.4%) at 30%, native whey protein concentrate (Nutrilac QU7560, ex Arla), potassium sorbate at 0.1% and demineralised water. The mix was then heated to 50 °C in about 20 minutes, subsequently homogenized in an APV Lab1000 homogeniser at 300 bar, and then packed in 100 ml tubs (6.5 cm diameter), sealed and placed for storage in a refrigerator at 5 °C. The product has a pH of 6.8.

Comparative Example -B:

A product as per Comparative Example A was prepared except that the sample was acidified using a 50% citric acid solution in demineralised water to a pH of 4.5 after it was homogenised but before being packed.

Comparative Example-C:

A premix as in comparative example A was first prepared. The premix was heated to a temperature of 85 °C in about 20 minutes. The heated mixture was then homogenized in an APV Lab1000 homogeniser at 300 bar. The sample was then acidified to a pH of 4.5 using a 50% citric acid solution in demineralised water. The product was then packed in 100 ml tubs (6.5 cm diameter), sealed and placed for storage in a refrigerator at 5 °C.

Comparative Example-D

A premix as in comparative example A was first prepared. The premix at a temperature of 50 °C was first homogenized in an APV Lab1000 homogeniser at 300 bar. The sample was then heated to a temperature of 85 °C in about 20 minutes. The product having a pH of 6.8 was then packed in 100 ml tubs (6.5 cm diameter), sealed and placed for storage in a refrigerator at 5 °C.

Comparative Example -E:

A product as per Comparative Example C was prepared except that the partly crystalline fat blend in the composition was replaced by (liquid) sunflower oil.

Example-1

A premix as in comparative example A was first prepared. The premix at a temperature of 50 °C was first homogenized in an APV Lab1000 homogeniser at 300 bar. The sample was then heated to a temperature of 85 °C in about 20 minutes and then acidified to a pH of 4.5 using a 50% citric acid solution in demineralised water. The product was then packed in 100 ml tubs (6.5 cm diameter), sealed and placed for storage in a refrigerator at 5 °C.

The samples were stored in the refrigerator at 5°C for one week and then subjected to experiments of cycling temperature changes that involved three cycles, each cycle consisting of 20 hours at 5 °C and 4 hours at 25 °C. At the beginning and the end of the three cycles, the samples were then measured for the $d_{3,2}$ value and the firmness values, as hereinabove described. The measured values are summarized in Table-1.

**Table-1**

| Sample | $d_{3,2}$ start □m | $d_{3,2}$end □m | Firmness, start, g | Firmness, end, g |
|---|---|---|---|---|
| Comp. Exp -A | 0.86 | 0.92 | 0 | 0 |
| Comp. Exp -B | 0.89 | 1.51 | 43 | 120 |
| Comp. Exp -C | 0.95 | 1.85 | 355 | 970 |
| Comp. Exp -D | 1.09 | 1.02 | 0 | 0 |
| Comp. Exp -E | 0.75 | 0.71 | 65 | 72 |
| Example -1 | 1.10 | 1.15 | 580 | 526 |

The data in Table-1 indicates that the process as per the invention provides for a firm emulsion that retains its desired properties when subjected to cycling temperature changes and this is not achieved with the processes of the prior art.

**Claims**

1. Process for the preparation of a water continuous acidified emulsion comprising 10 to 50% partly crystalline fat and protein, said process comprising homogenizing, heating and acidifying, characterized in that the process comprises the sequence of
   (a) Homogenising, at a temperature less than 60 °C, a water continuous emulsion comprising fat and protein where the percentage denatured protein is less than 20%
   (b) heating to a temperature and for sufficient time to prepare an emulsion with percentage denaturation of protein of more than 20% and
   (c) acidifying to a pH less than the pH of gelling of the protein having the highest pH of gelling in the emulsion.

2. Process according to claim 1 wherein the emulsion before homogenisation is prepared by mixing fat and protein, in an aqueous phase where the percentage denatured protein is less than 20%.

3. Process according to claim 2 wherein the fat is a vegetable fat.

4. Process according to any of claim 1 to 3 wherein the product of step (b) is acidified to a pH in the range of 5.8 to 3.8.

5. Process according to any of claims 1 to 4 wherein the step (c) is carried out using one or more food grade chemical acidifying agent.

6. Process according to claim 5 wherein the chemical acidifying agent is chosen from citric acid, hydrochloric acid or acetic acid.

7. Process according to any of claims 1 to 6 wherein the emulsion in step (b) is heated to a temperature higher than 70 °C.

8. Process according to any of claims 1 to 7 wherein the emulsion in step (b) is heated for 2 to 120 minutes.

9. A water continuous acidified emulsion obtainable by the process according to any of claims 1 to 8.

10. A water continuous acidified emulsion comprising from 10 to 50 % fat, and from 0.1 to 6 %protein, having a pH in the range of 5.8 to 3.8 wherein the $d_{3,2}$ value, as herein described, does not change by more than 25% over three temperature cycles, each cycle consisting of four hours at 25 °C and twenty hours of 5 °C.

11. A product according to claim 9 or 10 wherein the solid fat content is between 40% and 99% at 5 °C, and between 0.1 and 10% at 25 °C.

12. A product according to any of claims 9- 11 wherein the firmness value of the product, as herein described, does not change by more than 25 percent over three temperature cycles, each cycle consisting of four hours at 25 °C and twenty hours of 5 °C.

13. A process according to any of claims 1 to 8 or a product according to any of claims 9 to 12 wherein the protein is a whey protein.

**ABSTRACT**

The invention relates to a process for preparing water continuous acidified emulsions that can be used as a spread on food products including bread or toast or as a semi-solid acidified cream. The process comprises the steps of homogenising an emulsion comprising 10 to 50% fat and protein at a low temperature followed by heating such that the percentage denatured protein is greater than 20% followed by the step of acidification. The product so produced is stable to repeated cyclic temperature changes that the product undergoes between when it is stored in the refrigerator at about 5 °C and when it is taken out for use when it warms up to ambient temperatures of about 25 °C. The invention also relates to a stable water continuous acidified emulsion.

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